BIOMARKER DISCOVERY WITH SINGLE MOLECULE COUNTING (SMC™) TECHNOLOGY

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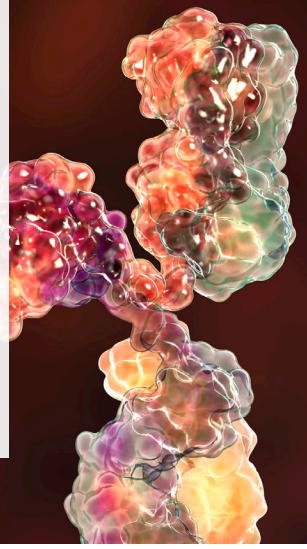
BIOMARKERS SHAPE OUR UNDERSTANDING OF PATHOLOGY



Biomarkers are objectively measurable characteristics that serve as reflections of biological states or signs of specific conditions. Many different types of biomarkers exist, ranging from disease-specific mutated nucleic acid sequence to elevations in body temperature. However, the vast majority of biomarkers found in the biological sciences, and in disease research specifically, are proteins. Proteins are popular as biomarkers for two reasons. First, their ability to directly modulate biological mechanisms means that protein-derived data is highly reflective of—and relevant to—pathophysiological status. Second, they are relatively easy to detect and quantify—something that has been largely facilitated by the advent of antibody-based immunoassays.

The History of Immunoassays

The first immunoassay was designed by Solomon Berson and Rosalyn S. Yalow in 1959. They developed a simple radioimmunoassay where radioactive iodine-labeled insulin and unlabeled insulin competed to bind to an antibody. The measured amount of radioactivity determined sample concentrations.[1] Since this inception, immunoassay technology has improved by leaps and bounds. Monoclonal antibody production, first explored in 1975 and refined over subsequent years, allowed for the development of non-competitive (sandwich) immunoassays. This, in tandem with the concurrent development of non-radioactive labels, made immunoassays less dangerous, easier to use, and above all, more accurate and sensitive.[1] Immunoassay technologies have only continued to evolve since that point; today, scientists use them as front-line clinical diagnostic tools,[2] and as critical components of almost all aspects of research, from basic discovery to drug development.[3]

Immunoassays Enable Biomarker Discovery

Researchers have recently become particularly interested in early disease detection, as the ability to identify signs of nascent pathogenesis offers tremendous advantages for combating many chronically debilitating or fatal illnesses, including cancer and neurodegenerative disorders.[4] For example, discovering new biomarkers for rheumatoid arthritis would offer novel methods for disease detection, which currently relies largely on symptom presentation. It would also provide insights on disease progression mechanisms and thereby aid in predicting patient prognosis.[5] Similarly, a host of biomarkers, ranging from fluid-based molecules to nucleic acids, have been identified for neurodegenerative disorders such as Alzheimer's disease.[6] These biomarkers not only provide insights into mechanistic causes for pathogenesis, they also hold potential for use in disease screening, as multiplex immunoassays can flag non-normal biomarker levels, resulting in additional investigation and possibly early intervention.[5,6]

Biomarker discovery is also a focus for researchers looking for ways to enable early detection of cardiovascular disease (CVD). CVD is best treated through prevention and early mitigation rather than post-hoc intervention. As such, scientists are looking for ways to not only detect and quantify asymptomatic cardiovascular pathology, but also to identify and stratify high-risk individuals.[7] A host of biomarkers, including classical indicators of cardiomyocyte damage, natriuretic and metabolic-related hormones, and circulating inflammatory markers, are now linked with cardiovascular disease onset and progression.[7] Importantly, protein biomarkers can also be used to identify congenital cardiovascular disorders prior to symptom onset. An immunoassay for N-terminal-prohormone B-type natriuretic peptide (NT-proBNP) accurately identified congenital heart disease in newborns as early as two days after birth.[8]

Immunoassays have helped shape perspectives on health and disease within the research and medical communities for years. As scientists delve deeper into the mechanisms of health and disease, they continue to look for new biomarkers that can offer a more comprehensive understanding of pathogenesis, either on their own or in tandem with already established biomarkers. To this end, immunoassay technology must continue to advance so that disease researchers have the tools that allow them to reach their goals and ambitions.

See references on page 7

INTRODUCING SINGLE MOLECULE COUNTING (SMC™) TECHNOLOGY

The enzyme-linked immunosorbent assay (ELISA), first discovered in 1971, is still the most commonly used technique for single target biomarker analysis. However, immunoassay technology progressed such that two distinct trajectories emerged. The first strives for better screening capabilities, which led to advances such as Luminex® technology and MILLIPLEX® assays to facilitate multiplex screening endeavors. The second continuously seeks to improve single target analysis sensitivity in order to uncover novel information about biomarkers of interest in a precise and flexible manner.[1] Ultimately, this desire has culminated in the Single Molecule Counting (SMC™) technology.

What SMC™ Technology Offers

SMC™ technology, including SMC™ immunoassay kits and the SMCxPRO™ platform, provides higher sensitivity protein quantification capabilities than standard multiplexing assays or ELISAs. Not only does SMC™ technology offer precision readings at femtogram/mL (rather than traditional picogram/mL) concentrations, it also possesses a larger dynamic range (4-5 logs) than standard multiplex assays (3-4 logs) or ELISAs (2-3 logs). On top of that, SMC™ technology consumes smaller sample volumes, allowing scientists to delve deeper into their precious specimens, and perform more experiments with them. SMC™ assays are for research use only and not for use in diagnostic procedures.

SMC™ technology uses traditional ELISA technology as a foundation. First, as with ELISA, analytes of interest are captured by bead or plate-bound primary antibodies. After this binding, unbound proteins are washed away, and a second fluorescently-labeled detection antibody is introduced. What results is a sandwich-based immunoassay complex. However, at this stage, rather than reading reporter signal immediately, the complexes are chemically disassociated into an eluate (containing the detection antibody and analyte), which is then transferred to a plate for signal reading. Here, a confocal laser systematically excites fluorescent-tagged antibodies present within the eluate well, and resulting signals are captured using an avalanche photodiode. Combined with the low background noise facilitated by the assay procedure, this allows for greater detection sensitivity and broader dynamic ranges. The latter allows for samples with greatly differing values—baseline controls, diseased samples, and post-treatment timepoints, for example—to be probed within the same experimental run, removing issues with inter-run variation.

In contrast to traditional ELISA, SMC™ technology can be used in tandem with data from multiplex biomarker screens, whether to confirm a result or to further investigate specific analytes of interest. Finally, SMC™ technology is highly flexible, allowing scientists to develop custom application-specific immunoassays in either bead or plate-based assay formats. All it takes is selecting the appropriate antibodies for conjugation and labeling. Here, we offer not only advice and troubleshooting, but also development, verification, and sample testing services.

SMC™ Technology Reshapes Scientific Thinking

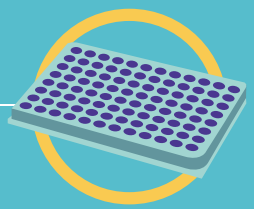
SMC™ technology allows researchers to identify previously undetectable biomarkers, as well as detect smaller shifts in biomarker levels over greater ranges. This information turns "yes-no" models into "high-low" models. Test results reveal values along a gradient, potentially conveying information about the presence or absence of disease, along with future risk, duration, and progression. SMC™ technology transforms how the scientific community understands, interprets, and uses biomarkers, offering great potential for disease research.

See references on page 7

peering beneath the waves

Sensitive assays can reveal a lot of "hidden" information to help identify new biomarkers, clarify how biomarker values relate to pathophysiological states and mechanisms, and shift understanding of disease progression and management.

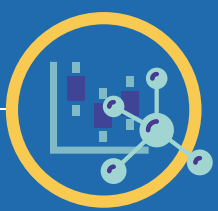
Established Biomarkers



Biomarkers indicate pathophysiological changes in disease progression. Biomarker levels detected by traditional ELISAs are useful indicators of cellular and systemic responses in disease models.

Unknown Biomarkers and Biomarkers of Low Abundance

Low abundance biomarkers can indicate early-onset disease prior to symptom manifestation, identifying potential therapeutic targets.



Gradual shifts in biomarker levels can indicate disease progression, and can provide important information for translational research and pharmacological testing.

Identifying small changes in low abundance biomarkers can help researchers identify "tipping points" for the onset of pathological mechanisms.

Biomarkers, especially low abundance biomarkers, may offer insights into factors affecting susceptibility to specific conditions.

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SMC™ TECHNOLOGY DRIVES DISEASE RESEARCH FORWARD

SMC™ technology is not just about detecting smaller biomarker concentrations, it is about translating that capability into tangible scientific advances and clinical approaches. To that end, SMC™ technology helps researchers better understand how biomarkers link to the pathophysiological processes that they represent, and allows them to find new therapeutic uses for biomarker-based data.

An Expanded Role for Cardiac Troponin I in Cardiovascular Disease

Cardiac troponin I (cTnI) is a classical biomarker for cardiomyocyte damage that is commonly used to assess the presence or absence of an event leading to myocardial necrosis.[1] However, as troponin detection technology improved, researchers found that circulating cTnI is present at very low concentrations in healthy individuals. More importantly, the "healthy" circulating cTnI range is distinctly lower than circulating cTnI levels in individuals experiencing—or who recently experienced—potentially asymptomatic cardiomyocyte stress events such as transient ischemia, myocardial inflammation, or even chronic strenuous exercise.[1-3] Critically, small increases in circulating cTnI levels can represent considerable elevations in risk for future cardiac events. A 2012 study noted that increases as little as 1 pg/mL—well below the 1.5 ng/mL positivity threshold used prior to the advent of high-sensitivity cTnI assays[1]—were associated with increased risk of cardiovascular event incidence.[4] SMC™ cTnI assays have a lower limit of detection value of 0.1 pg/mL, with a lower limit of quantification value of 0.69 pg/mL, making them well suited for identifying clinically relevant cTnI level differences, even at very low concentration ranges. The information provided by SMC™ cTnI assays can help researchers better understand the mechanisms involved in cTnI release and how they translate to elevated cardiovascular disease risk.[5]

Identifying and Detecting New Biomarkers for Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial joint inflammation and eventual destruction of cartilage and bone. The disease is heterogeneous in presentation, which makes it hard to detect during early stages. As such, research has focused on finding new RA biomarkers for generating more accurate prognostic tests.[6] A small cohort study using SMC™ technology identified seven potential biomarkers that showed altered levels in RA patients. Of these seven, IL-17A and IL-17F were particularly noteworthy. The former can be found in difficult to obtain synovial fluid, but previously had not been detectable in blood or serum owing to poor immunoassay sensitivity. Meanwhile, the latter has been identified not only as a biomarker for RA, but also as a potential therapeutic target for RA.[7] The demonstrated ability of SMC™ technology to discern both low biomarker concentrations and small shifts upon pathogenesis makes it potentially vital for predicting RA risk and identifying the mechanisms underlying RA onset.

Linking Skeletal Muscle Injury with Systemic Inflammation

Skeletal muscle injury can sometimes be sidelined as an easily rectified problem brought on by localized over-exertion. However, there is a relationship between physiological responses to muscle injury and systemic inflammatory processes.[8] This relationship is poorly defined at present, and inconsistencies in immunoassay-derived data, possibly caused by discrepancies between commercial kits, is a key reason for that. There is a need in this area for not only high assay sensitivity, but also assay designs that allow larger numbers of distinct molecules to be probed per experimental run.[8]

A recent study from the University of North Texas devised a unique approach for characterizing the inflammatory effects of exercise-induced skeletal injury. First, they ran a high-sensitivity MILLIPLEX® assay screening 21 inflammatory cytokines, and found nine elevated at either 4 or 24 hours—and six elevated at both timepoints—after muscle injury induced by extreme aerobic exercise. They then further probed IL-1β, IL-6, and TNF-α because the prior literature for these three cytokines was inconsistent, and did not observe elevated levels using the multiplex assay. In contrast with MILLIPLEX® results, the added sensitivity offered by an SMC™ assay revealed that all three of these cytokines were elevated at the 4 hour timepoint relative to their levels at 24 hours post-exercise.[8] Ultimately, the idea of combining these two assay strategies created a more comprehensive short-term and long-term map of post-exercise inflammatory responses.

See references on page 7

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Article 1 – Biomarker Discovery with Single Molecule Counting (SMC™) Technology

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Article 2 – Introducing Single Molecule Counting (SMC™) Technology

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Article 3 – SMC™ Technology Drives Disease Research Forward

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SMCxPRO™ Immunoassay Instrument and Kits

When the success of your research requires identifying and quantifying low-level proteins, you need technology you can rely on to detect your target. With the SMCxPRO™ platform you can detect extremely low levels of established disease biomarkers, capturing concentrations down to the femtogram/mL level, and as a result, monitor small changes in protein concentrations to accurately measure biomarkers associated with disease progression.

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